Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific GeneJET RNA Purification Kit

#K0731, #K0732

⚠ Read Storage information (p. 2) before first use!

www.thermoscientific.com/onebio

#__
Lot __
Expiry Date ___

CERTIFICATE OF ANALYSIS

The Thermo Scientific GeneJET RNA Purification Kit is qualified by isolating total RNA from 20 mg of mouse muscle or heart following the described protocol. The purified RNA has an $A_{260/280}$ ratio of ≥1.9. The RNA integrity numbers (RIN) of RNA purified from mouse muscle and heart are >7 and >8, respectively. Functional quality of the purified RNA is evaluated by RT-PCR of mouse dystrophin RNA resulting in a 13.3 kb PCR product.

Quality authorized by: Jurgita Zilinskiene

Rev.8 71

CONTENTS page

COMPONENTS OF THE KIT

GeneJET RNA Purification Kit	50 preps #K0731	250 preps #K0732
Proteinase K	600 µL	5 × 600 µL
Lysis Buffer	40 mL	200 mL
Wash Buffer 1 (concentrated)	40 mL	200 mL
Wash Buffer 2 (concentrated)	23 mL	100 mL
Water, nuclease-free	30 mL	125 mL
GeneJET RNA Purification Columns pre-assembled with Collection Tubes	50	250
Collection Tubes (2 mL)	50	250
Collection Tubes (1.5 mL)	50	250

STORAGE

Proteinase K is stable at room temperature as long as not opened. After being opened it should be stored at -20°C. Other components of the kit should be stored at room temperature (15-25°C).

DESCRIPTION

The GeneJET™ RNA Purification Kit is a simple and efficient system for purification of total RNA from mammalian cultured cells, tissue, human blood cells, bacteria, yeast and insects.
The kit utilizes a silica-based membrane technology in the form of a convenient spin column, eliminating the need for tedious cesium chloride gradients, alcohol precipitation or toxic phenol-chloroform extractions.
RNA molecules longer than 200 nucleotides can be isolated with the GeneJET RNA Purification Kit in 15 minutes after the lysis step. The high-quality purified RNA can be used in a wide range of downstream applications including RT-PCR, RT-qPCR, Northern blotting and other RNA-based analyses. *See* Table 1 for typical total RNA yields from various sources.

PRINCIPLE

Samples are lysed and homogenized in Lysis Buffer, which contains guanidine thiocyanate, a chaotropic salt capable of protecting RNA from endogeneous RNases (1). The lysate is then mixed with ethanol and loaded on a purification column. The chaotropic salt and ethanol cause RNA to bind to the silica membrane while the lysate is spun through the column (2). Subsequently, impurities are effectively removed from the membrane by washing the column with wash buffers. Pure RNA is then eluted under low ionic strength conditions with nuclease-free water.

Table 1. Typical total RNA yields from various sources

Source	Quantity	Yield, µg
Mouse heart	20 mg	10-15
Mouse muscle	30 mg	8-10
Mouse lung	30 mg	25-30
Mouse kidney	30 mg	25-30
Mouse liver	30 mg	60-65
Mouse spleen	5 mg	10-15
Bacillus pumilis cells	1×10^9 cells	15-20
Escherichia coli cells	1×10^9 cells	25-30
HeLa cells	5×10^6 cells	35-40
Jurkat cells	5×10^6 cells	40-50
Cos7 cells	1×10^6 cells	20-25
Saccharomyces cerevisiae cells	4×10^8 cells	150-160

IMPORTANT NOTES

Buffer preparation and handling

Add the indicated volume of ethanol (96-100%) to **Wash Buffer 1** (concentrated) and **Wash Buffer 2** (concentrated) prior to first use:

	50 preps (#K0731)		250 preps (#K0732)	
	Wash Buffer 1	Wash Buffer 2	Wash Buffer 1	Wash Buffer 2
Concentrated wash buffer	40 mL	23 mL	200 mL	100 mL
Ethanol (96-100%)	10 mL	39 mL	50 mL	170 mL
Total volume	50 mL	62 mL	250 mL	270 mL

After the ethanol has been added, mark the check box on the bottle's cover to indicate the completed step.

- Before each RNA purification experiment, supplement the required amount of **Lysis Buffer** with β-mercaptoethanol or DTT. Add 20 µL of 14.3 M β-mercaptoethanol or 20 µL of 2 M DTT to each 1 mL volume of **Lysis Buffer** used.
- Check **Lysis Buffer** for salt precipitation before each use. Re-dissolve any precipitate by warming the solution at 37°C, then cool back down to 25°C before use.
- Wear gloves when handling the **Lysis Buffer** and **Wash Buffer 1** as these solutions contain irritants (*see* p. 17 for SAFETY INFORMATION) and are harmful if contacted with skin, inhaled or swallowed.

Avoiding ribonuclease contamination

RNA purity and integrity is essential for downstream applications. RNA can be degraded by RNase A, which is a highly stable contaminant found in any laboratory environment. Care must be taken not to introduce RNAses into RNA preparation, especially during the column wash with Wash Buffer 2 and elution steps.

General recommendations to avoid RNase contamination:

- Wear gloves when handling reagents and RNA samples, as skin is a common source of RNases. Change gloves frequently.
- Use sterile, disposable RNase-free pipette tips.
- Use appropriate reagents to remove RNase contamination from non-disposable items (pipettes, centrifuges) and work surfaces.
- Keep all kit components tightly sealed when not in use. After usage close bottles immediately.

Starting material handling and storage

- When purifying RNA from fresh samples, place the samples on ice immediately after harvesting. Proceed to lysis and homogenization as quickly as possible.
- When samples are obtained from sacrificed animals or cadavers, limit the time between death and sample collection to isolate high quality RNA.
- If RNA is not to be purified immediately after tissue collection, freeze the samples in liquid nitrogen and store at -70°C. Frozen tissue samples should not be allowed to thaw during handling or weighing.
- Animal and bacterial cells can be pelleted and stored at -70°C until required. However, for RNA purification from yeast cells using enzymatic lysis, only freshly harvested samples can be used.
- Blood sample collection and RNA purification from blood cells should be carried out within the same day. Samples should be stored at 4°C until use. Do not freeze blood samples.

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Pipettes and sterile, RNase-free pipette tips
- Vortex
- Microcentrifuge
- Microcentrifuge tubes
- Disposable gloves
- Equipment for sample disruption and homogenization (depending on the method chosen):
  - Mortar and pestle
  - Rotor-stator homogenizer
  - Blunt needle and syringe
- 14.3 M β-mercaptoethanol or 2 M DTT
- Ethanol (96-100%)

Buffers

- For mammalian cultured cells lysate preparation:
  PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4).
- For gram-positive and gram-negative bacteria lysate preparation:
  TE buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA) containing lysozyme (0.4mg/mL final concentration).
- For yeast lysate preparation:
  Yeast lysis buffer (1 M sorbitol, 0.1 M EDTA, pH 7.4. Add 0.1% -β-mercaptoethanol and 50 units of lyticase or zymolyase 20T just prior to use).
- For Proteinase K dilution:
  TE buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA).

RNA PURIFICATION PROTOCOLS

Protocols for RNA purification from mammalian cultured cells, tissues, insects, human blood, bacteria and yeast are described on p. 6-13.

A. Mammalian Tissue and Insect Total RNA Purification Protocol

Before starting:

- Supplement the required amount of Lysis Buffer with β-mercaptoethanol or DTT. Add 20 µL of 14.3 M β-mercaptoethanol or 2 M DTT to each 1 mL volume of Lysis Buffer required.
- Prepare the required amount of Proteinase K solution: dilute 10 µL of Proteinase K (included) to 590 µL of TE buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA).

Step	Procedure
1	Weigh the tissue (use up to 30 mg of fresh or frozen tissue), take the insect and disrupt the material by one of the following methods: a) Disruption using a mortar and pestle. Place up to 30 mg of tissue (use up to 10 mg of spleen tissue) or insect into liquid nitrogen and grind thoroughly with a mortar and pestle. Transfer the tissue or insect powder immediately into a 1.5 mL microcentrifuge tube (not included) containing 300 µL of Lysis Buffer supplemented with β-mercaptoethanol or DTT. Vortex for 10 s to mix thoroughly. Note • Transfer the tissue or insect powder to the Lysis Buffer as quickly as possible. Leaving the powder without with the Lysis Buffer can result in degraded RNA. • The grinded tissue or insect should be directly used for RNA purification and should not be stored. • All grinded material must be thoroughly mixed with the Lysis Buffer and should not left dry on the walls of the tube (this can cause degradation of RNA). Homogenize the lysate using a rotor-stator homogenizer or pass the lysate through a blunt 20-gauge needle fitted to an RNase-free syringe several times. b) Disruption and homogenization using a rotor-stator homogenizer. Place up to 30 mg of tissue (use up to 10 mg of spleen tissue) or insect into a suitably sized vessel for homogenization containing 300 µL of Lysis Buffer supplemented with β-mercaptoethanol or DTT. Disrupt the material immediately using a conventional rotor-stator homogenizer for 20-40 s or until the suspension is uniform.
2	Add 600 µl of diluted Proteinase K (10 µL of the included Proteinase K diluted in 590 µL of TE buffer). Vortex to mix thoroughly and incubate at 15-25°C for 10 min.
3	Centrifuge for 5 min (if lysate is prepared from <10 mg of starting material) or 10 min (if lysate is prepared from >10 mg of starting material) at ≥12000 × g. Transfer the supernatant into a new RNase-free microcentrifuge tube (not included).
4	Add 450 µL of ethanol (96-100%) and mix by pipetting.

Step	Procedure
5	Transfer up to 700 µL of lysate to the GeneJET RNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at ≥12000 × g. Discard the flow-through and place the purification column back into the collection tube. Repeat this step until all of the lysate has been transferred into the column and centrifuged. Discard the collection tube containing the flow-through solution. Place the GeneJET RNA Purification Column into a new 2 mL collection tube (included).
6	Add 700 µL of Wash Buffer 1 (supplemented with ethanol, *see* p. 3) to the GeneJET RNA Purification Column and centrifuge for 1 min at ≥12000 × g. Discard the flow-through and place the purification column back into the collection tube.
7	Add 600 µL of Wash Buffer 2 (supplemented with ethanol, *see* p. 3) to the GeneJET RNA Purification Column and centrifuge for 1 min at ≥12000 × g. Discard the flow-through and place the purification column back into the collection tube.
8	Add 250 µL of Wash Buffer 2 to the GeneJET RNA Purification Column and centrifuge for 2 min at ≥12000 × g. *Optional*. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET RNA Purification Column to a sterile 1.5 mL RNase-free microcentrifuge tube (included).
9	Add 100 µL of Water, nuclease-free (included) to the center of the GeneJET RNA Purification Column membrane. Centrifuge for 1 min at ≥12000 × g to elute RNA. Note • More than 90% of RNA is eluted during the first elution step. For maximum RNA yield repeat the elution step with an additional 100 µL of Water, nuclease-free. • If more concentrated RNA is required or RNA is isolated from a small amount of starting material (e.g., < 5 mg) the volume of Water, nuclease-free can be reduced to 50 µL for the first and second elution steps. Please be aware that smaller volumes of eluant may result in a smaller final quantity of eluted RNA.
10	Discard the purification column. Use the purified RNA for downstream applications or store RNA at -20°C or -70°C until use.

B. Mammalian Cultured Cells Total RNA Purification Protocol

Before starting:

- Supplement the required amount of Lysis Buffer with β-mercaptoethanol or DTT. Add 20 µL of 14.3 M β-mercaptoethanol or 2 M DTT to each 1 mL volume of Lysis Buffer required.

Step	Procedure
1	a) Suspension cells Pellet up to 1×10^7 cells in an appropriate centrifuge tube by centrifugation for 5 min at 250 × g. Discard the supernatant. Rinse the cells once with PBS to remove residual growth medium. Repeat centrifugation step and discard the supernatant. b) Adherent cells Remove the growth medium from the cells (use up to 5×10^6 cells). Rinse the cells once with PBS to remove residual medium. Remove and discard PBS. Detach the cells from the culture plate by scraping in an appropriate volume of PBS or by trypsinization. Transfer the cells into a microcentrifuge tube (not included) and pellet them by centrifugation for 5 min at 250 × g. Discard the supernatant. Note. Pelleted cells can be directly used for RNA isolation or stored at -70°C until use.
2	Resuspend the cells collected in step 1a or 1b in 600 µl of Lysis Buffer supplemented with β-mercaptoethanol or DTT. Vortex for 10 s to mix thoroughly. Note • If after the addition of Lysis Buffer the mixture becomes viscous and dense, homogenization of the sample is required. Homogenize the lysate for 30 s using a rotor-stator homogenizer or pass the lysate through a blunt 20-gauge needle fitted to an RNase-free syringe several times. Incomplete homogenization will result in a significant reduction of RNA yields. • If any cell debris is observed in the sample, centrifuge the tubes for 5 min at 14000 × g and transfer the supernatant into a new RNase-free microcentrifuge tube (not included).
3	Add 360 µL of ethanol (96-100%) and mix the sample by pipetting.
4	Transfer up to 700 µL of lysate to the GeneJET RNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at ≥12000 × g. Discard the flow-through and place the purification column back into the collection tube. Repeat this step until all of the lysate has been transferred into the column and centrifuged. Discard the collection tube containing the flow-through solution. Place the GeneJET RNA Purification Column into a new 2 mL collection tube (included).
5	Add 700 µL of Wash Buffer 1 (supplemented with ethanol, *see* p. 3) to the GeneJET RNA Purification Column and centrifuge for 1 min at ≥12000 × g. Discard the flow-through and place the purification column back into the collection tube.

Step	Procedure
6	Add 600 µL of Wash Buffer 2 (supplemented with ethanol, *see* p. 3) to the GeneJET RNA Purification Column and centrifuge for 1 min at ≥12000 × g. Discard the flow-through and place the purification column back into the collection tube.
7	Add 250 µL of Wash Buffer 2 to the GeneJET RNA Purification Column and centrifuge for 2 min at ≥12000 × g. *Optional*. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET RNA Purification Column to a sterile 1.5 mL RNase-free microcentrifuge tube (included).
8	Add 100 µL of Water, nuclease-free (included) to the center of the GeneJET RNA Purification Column membrane. Centrifuge for 1 min at ≥12000 × g to elute RNA. Note • More than 90% of RNA is eluted during the first elution step. For maximum RNA yield, repeat the elution step with an additional 100 µL of Water, nuclease-free. • If more concentrated RNA is required or RNA is isolated from a small amount of starting material (e.g., (e.g., ≤1×10^6 cells), the volume of the Water, nuclease-free can be reduced to 50 µL for the first and second elution steps. Please be aware that smaller volumes of eluant may result in a smaller final quantity of eluted RNA.
9	Discard the purification column. Use the purified RNA for downstream applications or store RNA at -20°C or -70°C until use.

C. Human Blood Cells Total RNA Purification Protocol

Before starting:

- Supplement the required amount of Lysis Buffer with β-mercaptoethanol or DTT. Add 20 µL of 14.3 M β-mercaptoethanol or 2 M DTT to each 1 mL volume of Lysis Buffer required.
- Blood sample collection and RNA purification from blood cells should be carried out within the same day. Samples should be stored at 4°C until use. Do not freeze blood samples.

Step	Procedure
1	Collect blood cells by centrifugation of 0.5 mL of whole blood at 400 × g for 5 min at 4ºC. Blood cells will generate a pellet of approximately 60-70% of the total sample volume. Remove the clear supernatant (plasma) from the pellet with a pipette.
2	Resuspend the pellet in 600 µL of Lysis Buffer supplemented with β-mercaptoethanol or DTT. Vortex or pipet to mix thoroughly.
3	Add 450 µL of ethanol (96-100%) and mix by pipetting.
4	Transfer up to 700 µL of lysate to the GeneJET RNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at ≥12000 × g. Discard the flow-through and place the purification column back into the collection tube. Repeat this step until all of the lysate has been transferred into the column and centrifuged. Discard the collection tube containing the flow-through solution. Place the GeneJET RNA Purification Column into a new 2 mL collection tube (included).
5	Add 700 µL of Wash Buffer 1 (supplemented with ethanol, *see* p. 3) to the GeneJET RNA Purification Column and centrifuge for 1 min at ≥12000 × g. Discard the flow-through and place the purification column back into the collection tube.
6	Add 600 µL of Wash Buffer 2 (supplemented with ethanol, *see* p. 3) to the GeneJET RNA Purification Column and centrifuge for 1 min at ≥12000 × g. Discard the flow-through and place the purification column back into the collection tube.
7	Add 250 µL of Wash Buffer 2 to the GeneJET RNA Purification Column and centrifuge for 2 min at ≥12000 × g. *Optional*. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET RNA Purification Column to a sterile 1.5 mL RNase-free microcentrifuge tube (included).
8	Add 50 µL of Water, nuclease-free (included) to the center of the GeneJET RNA Purification Column membrane. Centrifuge for 1 min at ≥12000 × g to elute RNA.
9	Discard the purification column. Use the purified RNA for downstream applications or store RNA at -20°C or -70°C until use.

D. Bacteria Total RNA Purification Protocol

Important Note

For RNA isolation bacteria cells should be harvested during the exponential phase of growth (OD_{600}=0.5-1). Do not use an overnight culture for RNA isolation.

Before starting:

- Supplement the required amount of TE buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA) with lysozyme (not included) to final concentration of 0.4 mg/mL.
- Supplement the required amount of Lysis Buffer with β-mercaptoethanol or DTT. Add 20 µL of 14.3 M β-mercaptoethanol or 2 M DTT to each 1 mL volume of Lysis Buffer required.

Step	Procedure
1	Transfer 1.5 mL of Gram-negative or Gram-positive bacterial culture (up to 1×10^9 cells) to a 1.5 mL microcentrifuge tube. Collect cells by centrifugation for 2 min at ≥12000 x g. Carefully remove the supernatant, leaving the pellet as dry as possible.
2	Resuspend the pellet in 100 µL of freshly prepared TE buffer supplemented with lysozyme (0.4mg/mL final concentration). Invert the tube several times to mix.
3	Incubate the resuspended cells for 5 min at 15-25°C.
4	Add 300 µL of Lysis Buffer supplemented with β-mercaptoethanol or DTT. Mix thoroughly by vortexing for about 15 s until a homogeneous mixture is obtained.
5	Add 180 µL of ethanol (96-100%) and mix by pipetting.
6	Transfer up to 700 µL of lysate to the GeneJET RNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at ≥12000 × g. Discard the flow-through and place the purification column back into the collection tube. Repeat this step until all of the lysate has been transferred into the column and centrifuged. Discard the collection tube containing the flow-through solution. Place the GeneJET RNA Purification Column into a new 2 mL collection tube (included).
7	Add 700 µL of Wash Buffer 1 (supplemented with ethanol, see p. 3) to the GeneJET RNA Purification Column and centrifuge for 1 min at ≥12000 × g. Discard the flow-through and place the purification column back into the collection tube.
8	Add 600 µL of Wash Buffer 2 (supplemented with ethanol, see p. 3) to the GeneJET RNA Purification Column and centrifuge for 1 min at ≥12000 × g. Discard the flow-through and place the purification column back into the collection tube.
9	Add 250 µL of Wash Buffer 2 to the GeneJET RNA Purification Column and centrifuge for 2 min at ≥12000 × g. *Optional*. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET RNA Purification Column to a sterile 1.5 mL RNase-free microcentrifuge tube (included).

(continued)

Step	Procedure
10	Add 100 µL of Water, nuclease-free (included) to the center of the GeneJET RNA Purification Column membrane. Centrifuge for 1 min at ≥12000 × g to elute RNA. Note • More than 90% of RNA is eluted during the first elution step. For maximum RNA yield repeat the elution step with an additional 100 µL of Water, nuclease-free. • If more concentrated RNA is required or RNA is isolated from a small amount of starting material (e.g., ≤1×10^6 cells) the volume of the Water, nuclease-free can be reduced to 50 µL for the first and second elution. Please be aware that smaller volumes of eluant may result in smaller final quantity of eluted RNA.
11	Discard the purification column. Use the purified RNA for downstream applications or store RNA at -20°C or -70°C until use.

E. Yeast Total RNA Purification Protocol

Important Note

For RNA isolation yeast cells should be harvested at the exponential phase of growth (OD_{600}=0.5-1). Do not use an overnight culture for RNA isolation. For cell disruption using enzymatic lysis (described below) use only freshly harvested cells.

Before starting:

- Prepare Yeast lysis buffer: 1 M sorbitol, 0.1 M EDTA, pH 7.4. Just prior to use add 0.1% β-mercaptoethanol and 50 units of lyticase or zymolyase 20T.
- Supplement the required amount of Lysis Buffer with β-mercaptoethanol or DTT. Add 20 µL of 14.3 M β-mercaptoethanol or 2 M DTT to each 1 mL volume of Lysis Buffer required.
- Prepare the required amount of Proteinase K solution: dilute 10 µL of Proteinase K (included) to 590 µL of TE buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA).

Step	Procedure
1	Transfer the yeast cell culture (up to 4×10^8 yeast cells) to a 1.5 mL microcentrifuge tube (not provided). Collect the cells by centrifugation for 2 min at ≥12000 × g. Discard the supernatant.
2	Resuspend the cell pellet in 100 µL of Yeast lysis buffer.
3	Incubate at 30°C for 30 min.
4	Add 300 µL of Lysis Buffer supplemented with β-mercaptoethanol or DTT. Mix thoroughly by vortexing or pipetting.
5	Add 600 µL of diluted Proteinase K (10 µL of the included Proteinase K diluted in 590 µL of TE buffer). Vortex to mix thoroughly and incubate at 15-25°C for 10 min.

Step	Procedure
6	Centrifuge for 10 min at ≥12000 × g. Transfer the supernatant into a new RNase-free microcentrifuge tube (not included).
7	Add 450 µL of ethanol (96-100%) and mix by pipetting.
8	Transfer up to 700 µL of lysate to the GeneJET RNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at ≥12000 × g. Discard the flow-through and place the purification column back into the collection tube. Repeat this step until all of the lysate has been transferred into the column and centrifuged. Discard the collection tube containing the flow-through solution. Place the GeneJET RNA Purification Column into a new 2 mL collection tube (included).
9	Add 700 µL of Wash Buffer 1 (supplemented with ethanol, *see* p. 3) to the GeneJET RNA Purification Column and centrifuge for 1 min at ≥12000 × g. Discard the flow-through and place the purification column back into the collection tube.
10	Add 600 µL of Wash Buffer 2 (supplemented with ethanol, *see* p. 3) to the GeneJET RNA Purification Column and centrifuge for 1 min at ≥12000 × g. Discard the flow-through and place the purification column back into the collection tube.
11	Add 250 µL of Wash Buffer 2 to the GeneJET RNA Purification Column and centrifuge for 2 min at ≥12000 × g. *Optional*. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET RNA Purification Column to a sterile 1.5 mL RNase-free microcentrifuge tube (included).
12	Add 100 µL of Water, nuclease-free (included) to the center of the GeneJET RNA Purification Column membrane. Centrifuge for 1 min at ≥12000 × g to elute RNA. Note • More than 90% of RNA is eluted during the first elution step. For maximum RNA yield repeat the elution step with an additional 100 µL of Water, nuclease-free. • If more concentrated RNA is required or RNA is isolated from a small amount of starting material the volume of Water, nuclease-free can be reduced to 50 µL for the first and second elution steps. Please be aware that smaller volumes of eluant may result in a smaller final quantity of eluted RNA.
13	Discard the purification column. Use the purified RNA for downstream applications or store RNA at -20°C or -70°C until use.

Genomic DNA Removal from RNA Preparations

Step	Procedure
1	Add to an RNase-free tube: RNA 1 µg 10X reaction buffer with $MgCl_2$ 1 µL DNase I, RNase-free (#EN0521) 1 µL (1 u) DEPC-treated Water (#R0601) to 10 µL Incubate at 37°C for 30 min. Note. Thermo Scientific RiboLock RNase Inhibitor (#EO0381), typically at 1 u/µL, can be included in the reaction mixture to prevent RNA degradation.
2	Add 1 µL 50 mM EDTA and incubate at 65°C for 10 min to inactivate the DNase I. Addition of EDTA is required as RNA hydrolyzes during heating with divalent cations in the absence of a chelating agent. Alternatively, repurify RNA using the GeneJET RNA Purification Kit (*see* p. 15 for RNA Cleanup Protocol) or perform a phenol/chloroform extraction.
3	Use the prepared RNA for downstream applications.

Note

- Do not use more than 1 u of DNase I per 1 µg of RNA.
- Volumes of the reaction mixture and 50 mM EDTA solution can be scaled up for larger amounts of RNA. The recommended final concentration of RNA is 0.1 µg/µL.

RNA Cleanup Protocol

GeneJET™ RNA Purification Kit can be used to clean up to 100 µg RNA cleanup after DNase I digestion or other enzymatic reactions.

Step	Procedure
1	Adjust the volume of the reaction mixture to 100 µL with Water, nuclease-free (included). Add 300 µL of Lysis Buffer without β-mercaptoethanol or DTT. Mix thoroughly by vortexing or pipetting.
2	Add 180 µL of ethanol (96-100%) and mix by pipetting.
3	Transfer the mixture to the GeneJET RNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at ≥12000 × g. Discard the collection tube containing the flow-through solution. Place the GeneJET RNA Purification Column into a new 2 mL collection tube (included).
4	Add 700 µL of Wash Buffer 1 (supplemented with ethanol, *see* p. 3) to the GeneJET RNA Purification Column and centrifuge for 1 min at ≥12000 × g. Discard the flow-through and place the purification column back into the collection tube.
5	Add 600 µL of Wash Buffer 2 (supplemented with ethanol, *see* p. 3) to the GeneJET RNA Purification Column and centrifuge for 1 min at ≥12000 × g. Discard the flow-through and place the purification column back into the collection tube.
6	Add 250 µL of Wash Buffer 2 to the GeneJET RNA Purification Column and centrifuge for 2 min at ≥12000 × g. *Optional*. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET RNA Purification Column to a sterile 1.5 mL RNase-free microcentrifuge tube (included).
7	Add 50 µL of Water, nuclease-free (included) to the center of the GeneJET RNA Purification Column membrane. Centrifuge for 1 min at ≥12000 × g to elute RNA. Note. For maximum RNA yield repeat the elution step with an additional 50 µL of Water, nuclease-free.
8	Discard the purification column. Use the purified RNA for downstream applications or store RNA at -20°C or -70°C until use.

TROUBLESHOOTING

Problem	Possible cause and solution
Low RNA yield	**Too much starting material was used for lysate preparation.** Reduce the amount of starting material. Do not use more tissue or cells than indicated in lysis protocol. **Starting material was not completely disrupted.** Reduce the amount of starting material. Increase disruption time. **Ethanol was not added to the lysate.** Make sure that ethanol was added to the lysate before applying the sample to the purification column. **Ethanol was not added to Wash Buffers 1 and 2.** Make sure that ethanol was added to Wash Buffers 1 and 2 prior to the first use. Follow instructions for Wash Buffer preparation on p. 3.
Degraded RNA	**Inappropriate handling of starting material.** When purifying RNA from fresh samples, place samples on ice immediately after harvesting. Proceed to lysis and homogenization as quickly as possible. Ensure that frozen samples are frozen in liquid nitrogen immediately after collection and stored at -70°C. Thawing of the samples should be avoided until addition of Lysis Buffer. **RNase contamination.** To avoid RNase contamination, wear gloves during all procedure and change gloves frequently. Use sterile, disposable RNase-free pipette tips. Remove RNase contamination from non-disposable items and work surfaces.
DNA contamination	Digest RNA preparation with DNase I (#EN0521). Inactivate DNaseI by heat treatment (in the presence of a chelating agent), phenol/chloroform extraction or re-purify RNA following RNA Cleanup Protocol (*see* p. 15).
Column clogging	**Too much starting material was used for lysate preparation.** Reduce the amount of starting material. Do not use more tissue or cells than indicated in lysis protocol. **Starting material was not completely disrupted.** Reduce the amount of starting material and increase disruption time. **Tissue debris was not removed before applying lysate on a column** (Mammalian Tissue and Insect Total RNA Purification Protocol). Centrifuge the disrupted and homogenized cell suspension to remove tissue debris prior to applying the lysate to the column.
Enzymatic reactions not running well	**Ethanol was carried over into the eluted RNA.** If residual solution is seen in the purification column after washing with Wash Buffer 2, empty the collection tube and re-spin the column for an additional 1 min at maximum speed (≥12000 × g). **Salt was carried over into the eluted RNA.** Use the Wash Buffers the correct order. Always wash the purification column with Wash Buffer 1 first and then proceed to wash with Wash Buffer 2.

SAFETY INFORMATION

Lysis Solution

Hazard-determining component of labeling: Guanidinium thiocyanate

Xn Harmful

Risk phrases

R22 Harmful if swallowed.
R36/38 Irritating to eyes and skin.

Safety phrases

S23 Do not breathe gas/fumes/vapour/spray.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37 Wear suitable protective clothing and gloves.
S60 This material and its container must be disposed of as hazardous waste.

Proteinase K

Hazard-determining components of labeling: Proteinase, Tritirachium album serine

Xn Harmful

Risk phrases

R42 May cause sensitization by inhalation.

Safety phrases

S23 Do not breathe gas/fumes/vapor/spray.
S36 Wear suitable protective clothing.
S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
S60 This material and its container must be disposed of as hazardous waste.

References

1. Chomczynski, P. and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162:156-159
2. Boom, R., C.J.A. Sol, M.M.M. Salimans, C.L. Jansen, P.M.E.W. Dillen, and J. van der Noordaa. 1990. Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol. 28:495–503.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.

© 2012 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.